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Leucine, glucose, and energy metabolism after 3 days of fasting in healthy human subjects[1-3]

K Sreekumaran Nair, MD, PhD; Paul D Woolf, MD; Stephen L Welle, PhD; and Dwight E Matthews, PhD

ABSTRACT Adaptations of leucine and glucose metabolism to 3 d of fasting were examined in six healthy young men by use of L-[1-^{13}C]leucine and D[6,6-$^{2}H_2$]glucose as tracers. Leucine flux increased 31% and leucine oxidation increased 46% after 3 d of fasting compared with leucine flux and oxidation after an overnight fast. Glucose production rate declined 38% and resting metabolic rate decreased 8% during fasting. Plasma concentrations of testosterone, insulin, and triiodothyronine were reduced by fasting whereas plasma glucagon concentrations were increased. We conclude that there is increased proteolysis and oxidation of leucine on short-term fasting even though glucose production and energy expenditure decreased. *Am J Clin Nutr* 1987;46:557–62.

KEY WORDS Fasting, leucine metabolism, energy metabolism, glucose metabolism

Introduction

The metabolic changes during a brief fast are different from those present in a prolonged fast. Conservation of energy and protein by the body during prolonged fasting has been demonstrated by reduced metabolic rate and urinary nitrogen excretion (1–3) and reduced leucine flux (proteolysis) (4, 5). During the first 3 d of fasting no significant changes in urinary nitrogen excretion and metabolic rate have been demonstrated (1, 3, 6–10). Forearm studies suggest an increased proteolysis after 2.5 d of fasting (11). However Sherwin (12) using physiologic infusions of unlabeled leucine could not demonstrate an increased leucine release from protein (proteolysis) after 3 d of fasting. Recently Tsalikian et al (13) using a tracer dilution technique demonstrated an increased leucine flux, indicating an increased proteolysis after 1.25 d of fasting. There are no data available on the effect of short-term fasting on leucine oxidation in man. Animal and in vitro experiments have demonstrated conflicting results on the effect of fasting on leucine oxidation (14–17).

Because of the conflicting data on the effect of short-term fasting on proteolysis and leucine oxidation, we undertook this study to investigate the effect of a 3-d fast on leucine flux (reflecting proteolysis) and leucine oxidation. We also measured endogenous glucose production because there are few data on the effect of short-term fasting on glucose production in nonobese subjects.

Subjects, materials and methods

Subjects

Six normal healthy male volunteers (age, 23.7 ± 2.4 y [mean ± SEM]; weight, 73.8 ± 3.7 kg; height, 1.77 ± .54 m; and weight/height^2 23.6 ± 1.56 kg/m^2) with no history or family history of diabetes mellitus were selected for this study after their informed consent was obtained. For many months before the study none of the subjects were on any special dietary regimen nor was there any documented weight loss for 1 wk before the study. The protocol for this study was approved by the Human Investigation Committee of the University of Rochester School of Medicine and Dentistry.

Materials

L-[1-^{13}C]leucine (99 atom percent excess), ^{13}C-sodium bicarbonate (98 atom percent excess), and D[6,6^{2}-H_2]glucose (98 atom percent excess) were purchased from Cambridge Isotopes Lab-

[1] From the Department of Medicine (KSN, PDW, SLW), Endocrine-Metabolism Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY and the Clinical Research Center (DEM), New York Hospital, Cornell Medical Center, New York, NY.

[2] Supported by NIH grants AM20494, AM25994, RR05403-23, RR-954, and RR00044.

[3] Address reprint requests to K Sreekumaran Nair, MD, PhD, Endocrine-Metabolism Unit, Monroe Community Hospital, 435 East Henrietta Road, Rochester, NY 14603-0905.

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oratories (Woburn, MA). Stock solutions of L-[1-^{13}C]leucine (10 mg/mL), ^{13}C-sodium bicarbonate (2 mg/mL), and D-[6,6^{2}-H_2]glucose (10 mg/mL) were prepared in sterile normal saline with sterile techniques. The solutions were filtered through a 0.22 micron millipore filter (Millipore Corp, Bedford, MA) and were tested for pyrogen before use. The solutions were injected into a sterile normal saline bag for continuous intravenous infusion. The bag was weighed before and after the infusion to calculate the rate of infusion. Total volume in the bag was calculated from the difference between total weight of bag with solutions and the empty bag. The undiluted stock solution was injected directly into the vein as priming dose.

Protocol

All subjects were admitted to the Clinical Research Center on day 1 (evening). They were taking their normal, unrestricted diet before admission. Leucine kinetics, glucose kinetics, indirect calorimetry, substrates, and hormones were measured after an overnight fast and after 3 d of fasting. During the fast the subjects were allowed acaloric fluids and electrolytes. Urine was collected for measurement of urinary total nitrogen (Kjeldahl method) (18) and urea nitrogen (enzymatic technique) (19).

Leucine and glucose kinetics and indirect calorimetry

On the morning of each infusion (after both overnight and 3-d fasts), a retrograde catheter was inserted into one dorsal hand vein and kept open by a normal saline infusion. This hand was kept in a warm box (maintained at 70°C) from 30 min before the baseline blood sample until the end of the study. This catheter was used to draw arterialized venous blood samples (20). Another catheter was introduced into a forearm vein in the contralateral arm for infusion of labeled leucine and glucose. After baseline blood and expired air samples for isotopic analysis were drawn, pulse doses of L-[1-^{13}C]leucine (0.75 mg/kg), ^{13}C-sodium bicarbonate (0.2 mg/kg), and D-[6,6^{2}-H_2]glucose (2.5 mg/kg) were given to prime the respective pools (21). A continuous intravenous infusion of L-[1-^{13}C]leucine (0.75 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) and D-[6,6-$^{2}H_2$]glucose (2.0 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) was then given for 4 h with a volumetric infusion pump. Blood samples and expired air samples were collected for isotopic analysis at 15 min intervals from 150 min onwards until the end of the study. Urine samples from 30 min before the infusion until the end of the infusion were collected for measuring urinary total nitrogen excretion during the tracer kinetics studies.

Oxygen consumption and carbon dioxide production were measured using an open-circuit indirect calorimeter (22, 23). A polyethelene mask with a special device to collect expired air samples for isotopic analysis was used when indirect calorimetry was performed (4). Oxygen consumption, carbon dioxide production, respiratory quotient (RQ), and metabolic rate of 10-min averages were recorded. The indirect calorimetry was done for 60–90 min during the ^{13}C-leucine infusions. Metablic rate was determined as described earlier (24). Urinary total nitrogen excretion during the period of the infusion was used to calculate nonprotein RQ. The amount of carbohydrate and fat oxidized was calculated from nonprotein RQ and O_2 consumption using the equation of Lusk (25).

Measurements of isotopic enrichment and calculation of leucine and glucose kinetics

Plasma ^{13}C-leucine enrichment (atom percent excess) was measured by chemical ionization gas chromatographic mass spectrometry (21), expired air $^{13}CO_2$ (atom percent excess) was measured in an isotope ratio mass spectrometer (21), and enrichment of dideuteroglucose was measured in a gas chromatographic mass spectrometer (26).

Leucine flux (rate of appearance) was calculated from plasma ^{13}C-leucine enrichment at plateau and the rate of ^{13}C-leucine infusion (21, 27). Leucine oxidation rate was calculated from $^{13}CO_2$ enrichment at plateau, CO_2 production, and plasma ^{13}C-leucine enrichment (21, 27). Because the carboxyl group of leucine which carries the label (^{13}C) is either irreversibly lost to expired $^{13}CO_2$ or is incorporated into protein, it is assumed that the nonoxidative portion of leucine disappearance rate represents leucine incorporation into protein. This portion is referred to as leucine outflow to protein.

The glucose production rate (appearance rate and disappearance rate) was calculated from plasma enrichment of dideuteroglucose at plateau (26). Glucose metabolic clearance (mL $\cdot$ kg^{-1} $\cdot$ min^{-1}) was calculated from glucose production rate (mg $\cdot$ kg^{-1} $\cdot$ min^{-1}) and plasma glucose concentration (mg/mL).

Glucose was measured by autoanalyzer (Beckman Instruments, Fullerton, CA), hydroxybutyrate by microfluoremetric enzymatic technique (28), and plasma free fatty acid by colorimetric titration (29). Insulin was measured using a commercial kit (Cambridge Nuclear, Billerica, MA), glucagon was measured using 30 k antiserum (30), testosterone and thyroid hormones were measured with radioimmunoassay kits (Radioassays System Laboratories, Carson, CA and Clinical Assays, Cambridge, MA, respectively), and epinephrine was measured by radioenzymatic assay (31). Plasma amino acid concentrations were measured by HPLC with fluorometric detection (32).

All results are expressed as mean ± SEM and statistical analyses were done by two-tailed paired t test. We also calculated mean ± SEM of changes from the postabsorptive state to the 3-d fasted state.

Results

The weights of the subjects decreased from 73.8 ± 3.7 kg to 70.1 ± 3.0 kg after 3 d of fasting ($p < 0.01$). The daily urinary nitrogen excretion (total and urea) is given in **Table 1.** The mean of total urinary nitrogen excretion and urinary urea nitrogen showed a tendency to decrease from the first 24 h after fasting when the fast progressed. However the change in urinary nitrogen (total and urea) loss was not statistically significant.

Resting metabolic rate and the amounts of carbohydrate, protein, and fat oxidized during the period of leucine and glucose kinetic studies after an overnight fast and after a 3-d fast are given in **Table 2.** There was an 8% average decrease in resting metabolic rate ($p < 0.05$). The percent contribution of carbohydrate and protein metab-

TABLE 1
Total urinary nitrogen (TUN) and urinary urea nitrogen (UUN) excretion during 3-day fast (mean ± SEM)

	Day 1	Day 2	Day 3
TUN (mmol/24 h)	795.17 ± 81.37	601.73 ± 82.80	703.80 ± 133.48
UUN (mmol/24 h)	576.75 ± 124.20	493.24 ± 117.06	461.83 ± 72.09

TABLE 2
Effect of 3-d fast on resting metabolic rate (RMR) and amounts of protein, carbohydrate, and fat oxidized (mean ± SEM)

	Postabsorptive state (A)	After 3-d fast (B)	Change from A to B
RMR (kcal/h)	73.5 ± 3.5	67.8 ± 3.3†	−5.7 ± 1.9
Protein oxidation* (g/h)	3.9 ± 0.3	3.4 ± 0.3	−0.5 ± 0.2
Carbohydrate oxidation‡ (g/h)	3.1 ± 0.9	1.5 ± 0.5†	−1.6 ± 0.5
Fat oxidation‡ (g/h)	5.1 ± 0.5	5.7 ± 0.3†	0.7 ± 0.2

* From total urinary nitrogen excretion.
† Significantly different from postabsorptive state, $p < 0.05$.
‡ From nonprotein RQ.

olized for energy fell from 17 ± 5 to 9 ± 2% ($p < 0.05$) and from 21 ± 1 to 19 ± 1% (NS), respectively, while the contribution of fat increased from 61 ± 4 to 75 ± 3% ($p < 0.05$).

Plasma glucose concentrations decreased from 92 ± 3 to 68 ± 6 mg/dL (5.11 ± 0.17 to 3.77 ± 0.33 mmol/L; $p < 0.01$), free fatty acid concentrations increased from 0.47 ± 0.07 to 0.99 ± 0.11 mmol/L ($p < 0.05$), and β-hydroxybutyrate levels increased from 0.17 ± 0.09 to 2.53 ± 0.27 mM ($p < 0.001$). Blood urea did not change significantly during fasting.

Table 3 indicates hormone levels. There was a significant decrease of serum T_3, plasma insulin, and plasma testosterone whereas plasma glucagon levels increased. There was no statistically significant change in serum free T_4, cortisol, and epinephrine levels.

There was a significant decrease in endogenous glucose production after 3 d of fasting (**Table 4**). The calculated metabolic clearance rate of glucose was reduced from 2.69 ± 0.12 to 2.24 ± 0.14 $mg \cdot kg^{-1} \cdot min^{-1}$ ($p < 0.01$).

Leucine flux and leucine oxidation increased in every subject (Table 4). However the percent of leucine oxidized ([leucine oxidation/leucine flux] × 100) did not change significantly from the overnight fast (17.6 ± 0.6%) during the 3-d fast (19.9 ± 1.3%). The calculated leucine outflow to protein increased from 71.9 ± 1.7 to 95.1 ± 2.4 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ ($p < 0.01$).

From the postabsorptive state to the 3-d fast, there was a significant decrease in plasma concentrations of glutamate, serine, alanine, glycine, threonine, arginine, and tyrosine whereas plasma concentrations of leucine and valine increased (**Table 5**).

Dicussion

This study demonstrates that leucine flux (reflecting proteolysis) increases in healthy young men after 3 d of fasting. Our results support studies of net amino acid balance across the forearm in 2.5-d fasted human volunteers, which demonstrated a net increase in leucine release (11). A recent study demonstrated that leucine flux increased from 87.6 ± 3.0 to 98.4 ± 6 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ (an 11% increase) after 1.25 d of fasting in healthy subjects (13). Our subjects fasted for 3 d and observed a greater increase (31%) in leucine flux.

Three days of fasting significantly increases leucine oxidation. Intracellular leucine enrichment, which is likely to be the immediate precursor of leucine oxidation, is better reflected by ketoisocaproate (KIC) enrichment (33); leucine oxidation could be an underestimation by ~15% because plasma leucine enrichment is ~15% higher than KIC enrichment (4, 33). In vivo rat (15) and in vitro tissue studies (14) demonstrated an increased leucine oxidation on fasting. However studies in rabbits (17) and dogs (16) demonstrated decreased leucine oxidation on short-term fasting. In humans restriction of carbohydrate intake increases leucine oxidation (34) whereas prolonged fasting decreases leucine oxidation (5).

The increased leucine flux and leucine oxidation observed in this study indicates increased protein catabolism, which was not reflected in the urinary total nitrogen excretion. No significant changes in urinary total nitrogen excretion were noted by other investigators during the first 3 d of fasting in man (1, 3, 6, 7, 9, 10). There are several explanations for why proteolysis and leucine oxidation can be elevated while nitrogen excretion is unchanged or even reduced. First, nitrogen excretion only provides information about the amounts of amino acids oxidized and gives no quantitative information about the rate of proteolysis. Thus if reincorporation of amino acids into protein is elevated along with an increase in proteolysis, net availability of amino acids for oxidation may not be increased. Second, increased oxidation of leucine does not imply that oxidation of all amino acids is increased. Even when proteolysis is elevated, a reduction in amino acid synthesis could reduce the availability of nonessential amino acids for oxidation. Finally, it has been suggested that the magnitude of urinary nitrogen loss on

TABLE 3
Effect of 3-d fast on hormone concentrations in six healthy subjects (mean ± SEM)

	Postabsorptive state	After 3-d fasting	Change from A to B
Serum T_3 (pmol/L)	1150.0 ± 119.8	844.8 ± 115.4*	−648.2 ± 102.9
Free T_4 (nmol/L)	18.0 ± 1.3	18 ± 1.3	0 ± 0.7
Insulin (pmol/L)	58.8 ± 1.4	43.1 ± 1.4*	−15.1 ± 2.9
Glucagon (pmol/L)	51.6 ± 7.8	93.4 ± 0.3*	41.2 ± 8.1
Cortisol (nmol/L)	298.0 ± 22.1	328.3 ± 30.3	−8.3 ± 41.4
Testosterone (nmol/L)			
Total	2492.1 ± 83.9	1843.8 ± 108.2*	−608.5 ± 254.8
Free	68.3 ± 8.7	37.8 ± 5.5†	−30.5 ± 11.1
Epinephrine (pmol/L)	196.5 ± 15.3	354.8 ± 43.1	157.5 ± 42.6

* Significantly different from postabsorptive state ($p < 0.05$).
† Different from postabsorptive state ($p = 0.055$).

TABLE 4
Effect of 3-d fast on steady-state plasma leucine flux, leucine oxidation, and percent leucine oxidation in healthy men

	Leucine flux		Leucine oxidation		Endogenous glucose production	
Subject	I*	II	I	II	I	II
	$\mu mol \cdot kg^{-1} \cdot h^{-1}$		$\mu mol \cdot kg^{-1} \cdot h^{-1}$		$\mu mol \cdot kg^{-1} \cdot min^{-1}$	
1	90.9	118.6	15.7	17.7	12.9	7.0
2	98.8	114.0	15.3	27.6	11.7	8.5
3	80.6	123.4	15.2	27.1	14.4	8.5
4	89.5	120.4	17.5	25.3	14.4	9.5
5	82.0	105.3	13.6	17.6	14.1	8.8
6	93.9	122.1	18.5	25.3	13.8	8.3
	89.3 ± 2.6	117.3 ± 2.5†	16.0 ± 0.7	23.4 ± 1.7†	13.55 ± 0.39	8.4 ± 0.33†

* I = overnight fast; II = after 3-d fast.
† II significantly different from I ($p < 0.01$).

the first day of fasting (especially in the postabsorptive state) depends on the protein intake on the previous day and that the predominant protein oxidation on this day is from labile protein (7). When the labile protein store is depleted, there is an increased degradation of structural proteins. If the leucine content of structural proteins is higher than that of labile protein, an enhanced leucine flux would be observed when structural protein breakdown increased. No correlation between leucine catabolism and urea production was observed in exercise studies in man (35), further demonstrating that leucine kinetics and nitrogen excretion do not always change in the same direction.

The increases in branched-chain amino acid levels and decreases in other amino acids during short-term fasting have been reported previously (12, 36). The increase in branched-chain amino acid levels is consistent with the increased proteolysis. The reduction in some of the other amino acids may be related to reduced amino acid synthesis (in the case of nonessential amino acids) or increased utilization of amino acids for gluconeogenesis. Although total glucose production was reduced, this does not mean that gluconeogenesis from amino acid was reduced since in the immediate postabsorptive state only a small fraction of total glucose production is from amino acid-derived gluconeogenesis.

TABLE 5
Effect of 3-d fast on plasma amino acid concentrations (μM)

	Overnight fast (A)	3-d fast (B)	Change from A to B
Alanine	296 ± 20	182 ± 16*	−113 ± 21
Arginine	70 ± 6	46 ± 6*	−24 ± 5
Asparagine	34 ± 3	29 ± 2	−5 ± 2
Glutamate	142 ± 12	110 ± 6*	−32 ± 6
Glutamine	349 ± 29	312 ± 29	−37 ± 15
Glycine	222 ± 17	167 ± 18	−25 ± 46
Histidine	70 ± 3	64 ± 3*	−5 ± 2
Phenylalanine	55 ± 3	53 ± 4	−2 ± 1
Serine	108 ± 4	88 ± 4*	−20 ± 4
Threonine	158 ± 11	108 ± 8*	−50 ± 10
Tyrosine	48 ± 4	42 ± 2	−6 ± 3
Lysine	183 ± 12	164 ± 18	−10 ± 14
Leucine	123 ± 9	210 ± 21†	86 ± 23
Valine	225 ± 13	315 ± 22†	90 ± 25

* Lower than after overnight fast, $p < 0.05$.
† Greater than after overnight fast, $p < 0.01$.

After 2.5 d of fasting, net splanchnic glucose output is reduced by an average of 63% in normal volunteers (37), which is similar to the 66% reduction in net splanchnic glucose output after 4 d of carbohydrate deprivation (38). However, we found that total-body glucose production was reduced by only 38% after 3 d of fasting, which is similar to the 36% reduction in total-body glucose production after 3 d of fasting in obese subjects (39). If we assume that fasting does not increase splanchnic glucose utilization, these data suggest that much of the glucose production after 3 d of fasting comes from extrasplanchnic gluconeogenesis. The most likely source of this extrasplanchnic gluconeogenesis is the kidney, which accounts for ~50% of the total gluconeogenesis during prolonged fasting (9).

An adaptive decrease in the metabolic rate on fasting of longer than 3 d was reported by Benedict (1) and other pioneering workers, as summarized by Lusk (7). We demonstrated a small but consistent decrease in resting metabolic rate in our normal subjects after a 3-d fast. During this period the contribution of carbohydrate oxidation for energy requirements decreased at the expense of increased fat utilization. Our measurements were done in steady-state conditions and therefore changes in gluconeogenesis and ketone metabolism should not affect our calculations of energy expenditure. Under nonsteady-state conditions, ketone metabolism and gluconeogenesis could cause errors in the calculation of energy expenditure if standard equations are used (40).

Plasma testosterone levels decreased significantly after 3 d of fasting. Chronic malnutrition is known to decrease plasma concentrations of testosterone (41, 42). Morbidly obese men undergoing a prolonged therapeutic fast did not have reduced testosterone concentrations (42) and testosterone levels in moderately obese men did not change until after 10 d of fasting (43). Moderate weight

loss on a low-calorie diet increased testosterone levels in another study (44). Our lean subjects reduced their plasma testosterone concentration after only 3 d of fasting. It is possible that the existing body energy stores are a determinant of the plasma testosterone concentration. Testosterone is known to increase lean body mass (45) but the effect of a decreased testosterone concentration on leucine flux and leucine oxidation is not known.

Decreased insulin levels and increased glucagon levels were observed previously after 3 d of fasting (8). An increased leucine flux was observed in insulin-deprived type I diabetic patients (27) and insulin causes a dose-related fall in leucine flux in postabsorptive man (46). Thus the reduced insulin levels along with insulin resistance during fasting (47) could be responsible for an increased leucine flux. Glucagon was shown recently to affect protein metabolism in animals (48) but its effect on protein metabolism in man has yet to be determined. The fall in serum T_3 and fasting-induced T_3 resistance (49) might have caused the fall in RMR but its effect on protein metabolism remains to be determined (4, 5, 50).

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